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Further notes on the morphology and distribution of *Neopaxillus echinospermus* (Agaricales, Basidiomycota) in Southern Brazil

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Abstract: *Neopaxillus echinospermus* is a common but poorly understood agaric species from South America (Argentina, Bolivia, Brazil and Paraguay). In this paper, we discuss conflicting morphological features reported in the literature and expand the distribution of the species to Seasonal Deciduous and Semideciduous Forests from Rio Grande do Sul and Paraná states, respectively, in Southern Brazil. Hyphal structure of the pileipellis, presence of cheilocystidia and caulocystidia, presence of clamp connections and oleiferous (thrombopleurous) hyphae, and the basidiospore morphology under scanning electron microscopy are illustrated and discussed in detail.

Key words: boletoid; Crepidotaceae; Seasonal Forest; Neotropical fungus; Paxillaceae

Neopaxillus echinospermus (Speg.) Singer is a mushroom species, with a limited and poorly documented distribution in South America, comprising Argentina, Brazil, Paraguay and Bolivia (Watling and de Meijer 1997). This agaric was originally described by Singer (1948) as *Neopaxillus echinosporus* Singer, based on specimens collected by J. Rick supposedly in Rio Grande do Sul, in Southern Brazil. This name was put into synonymy based on further examination of *Naucoria echinosperma* Speg. from Brazil (Spegazzini 1889), which was considered the correct basionym of the species; for a detailed nomenclatural discussion, see Watling and Aime (2013).

For many years, *Neopaxillus* Singer remained as a monotypic genus in the Crepidotaceae (Imai) Singer, until Singer and Lodge (1988) described *N. plumbeus* from Costa Rica, Central America. Based on both macro- and micromorphological similarities, some researchers suggested that the genus would be related to the gilled boletes, proposing its placement in the Paxillaceae Lotsy (Singer 1986) or Serpulaceae Jarosch & Bresinsky (Binder and Hibbett 2006). Recently, Vizzini et al. (2012) described *N. dominicanus* from Central America and their phylogenetic resulted in the return of *Neopaxillus* to the Crepidotaceae. Finally, Watling and Aime (2013) analyzed morphology and 28s ribosomal DNA region of *N. echinospermus* and reassured the position of the type species in the latter family.

In spite of the current and relatively consistent position of the genus, supported on molecular analysis, conflicting morphological data have been noted in the literature, such as the absence or presence of cystidia and clamp connections, and the hyphal structure of the pileipellis.

Thus, the aim of this paper is to provide modern and detailed descriptions of *N. echinospermus*, updating the morphological concept of the species, based on collections from Atlantic Forest in Southern Brazil, and to expand the known distribution of the fungus in Seasonal Semideciduous and Deciduous Forests of that biome.

Samples were collected, during recent fieldwork in Seasonal Semideciduous Forest from the western region of Paraná state, and previous collections in Seasonal Deciduous Forests from central region of Rio Grande do Sul state. These ecosystems are two common vegetation types in the Atlantic Forest biome of Southern Brazil, in which a portion of the trees defoliates during the dry season (IBGE 2012). Moreover, these forests are characterized by the presence of tree species of Anacardiaceae, Apocynaceae, Bignoniaceae, Boraginaceae, Caricaceae, Fabaceae, Malvaceae, Meliaceae, Moraceae, Rutaceae and Sapindaceae (Roderjan et al. 2002).

Morphological analysis (both macro- and microscopical) followed standard procedures for agaricoid fungi (Singer 1986). Color names and codes adopted in the macroscopic features are from Kornerup and Wanscher

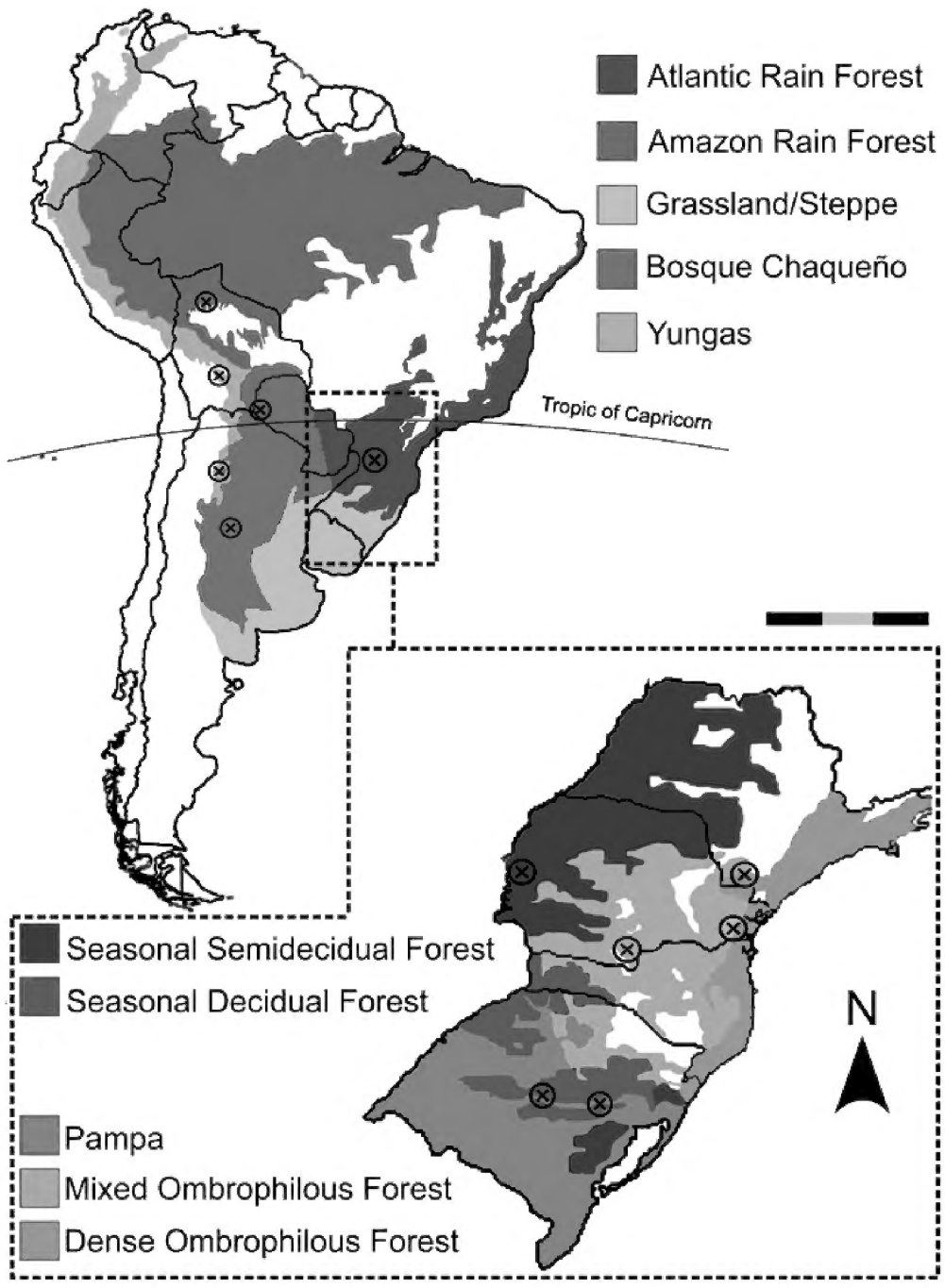


Figure 1. Known distribution of *Neopaxillus echinospermus*.

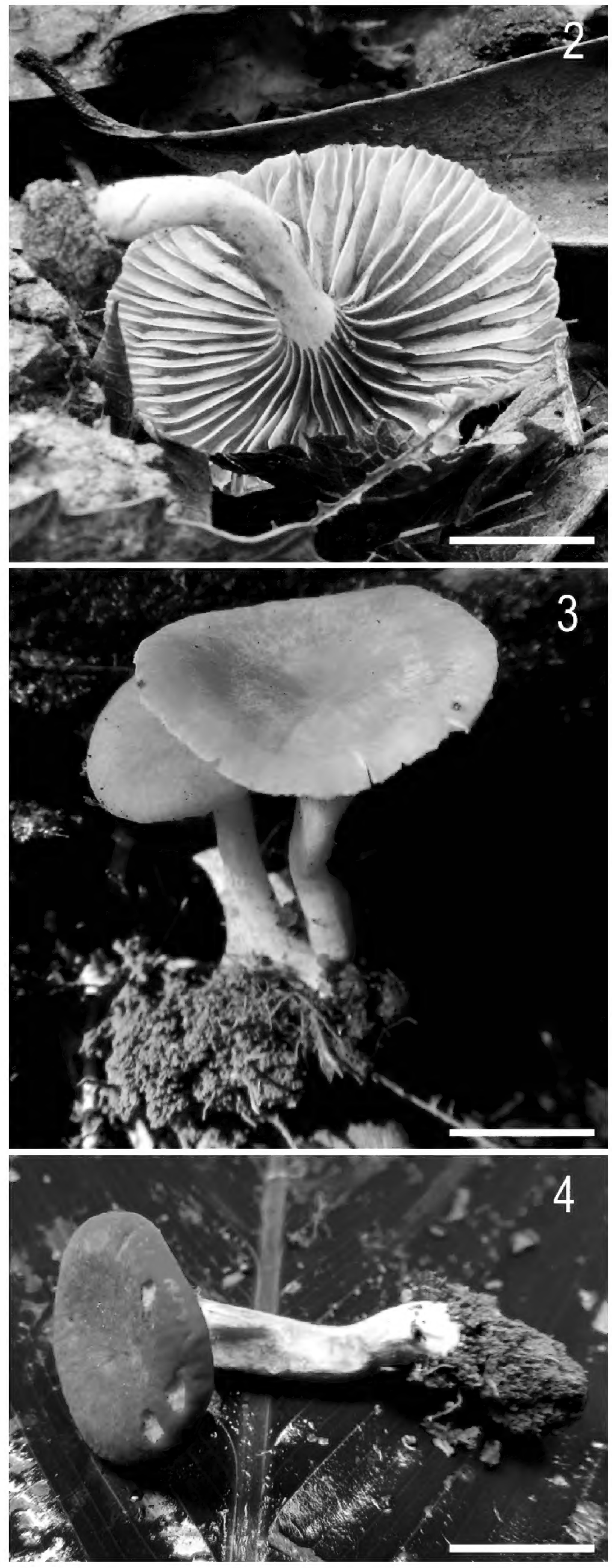
(1978). Micrographs were made from a Motic BA310 light microscope with a Moticam 2500 digital camera, and measurements were taken through software Motic Image Plus 2.0. In the basidiospores description Q is the quotient between the length and width, Qm is the medium value of Q, and n is the number of measured basidiospores/number of analyzed basidiomata/number of collections. Scanning electron micrographs (SEM) were performed at the Center of Electron Microscopy of the Federal University of Paraná at Curitiba (CME/UFPR), under a Jeol JSM-6360LV scanning electron microscope. Examined specimens are preserved at the herbaria HCP (Universidade Federal do Paraná, Campus Palotina) and HCB (Universidade de Santa Cruz do Sul, Departamento de Biologia e Farmácia).

Neopaxillus echinospermus (Speg.) Singer, Lilloa 22: 633, 1951 ("1949"). Figures 2–15

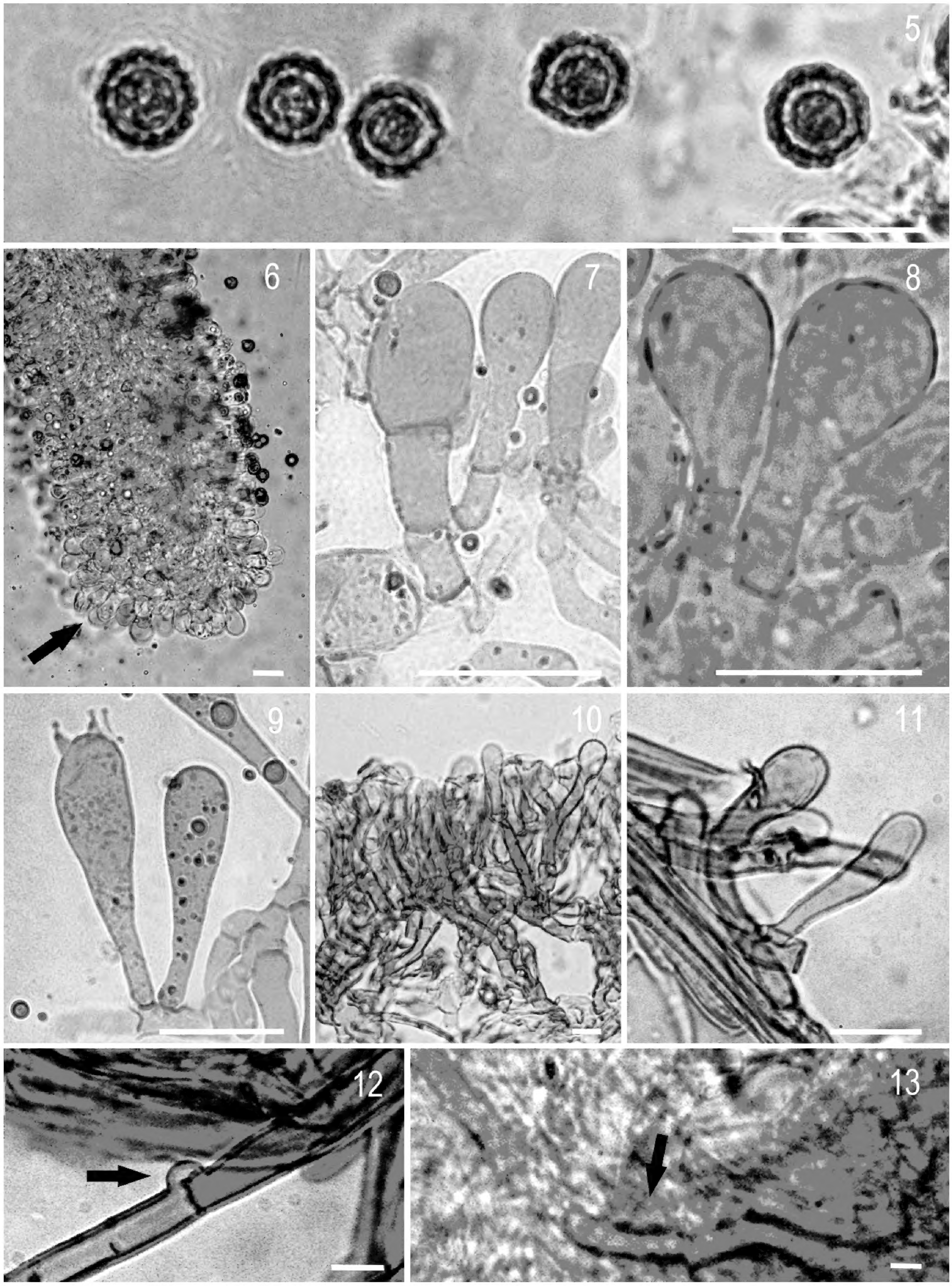
≡ *Naucoria echinosperma* Speg., Bol. Acad. Nac. Cienc. Cordoba 11: 424, 1889.

= *Neopaxillus echinosporus* Singer, Mycologia 40: 262, 1948.

Basidiomata agaricoid, omphalinoid. Pileus 12–36 × 9–34 mm diam., infundibuliform, plano-depressed or applanate, surface dry and smooth, hygrophanous, yellowish brown (5D8) golden brown (5D7), greyish yellow (4C6) to reddish orange (7A8) when fresh (Figure 3–4),



Figures 2–4. Basidiomata of *Neopaxillus echinospermus* (**2**, HCP 713, photo by G. Coelho; **3**, HCP 704 photo by A. Silva-Filho; **4**, HCP 706 photo by A. Silva-Filho). Scale bar = 25 mm.

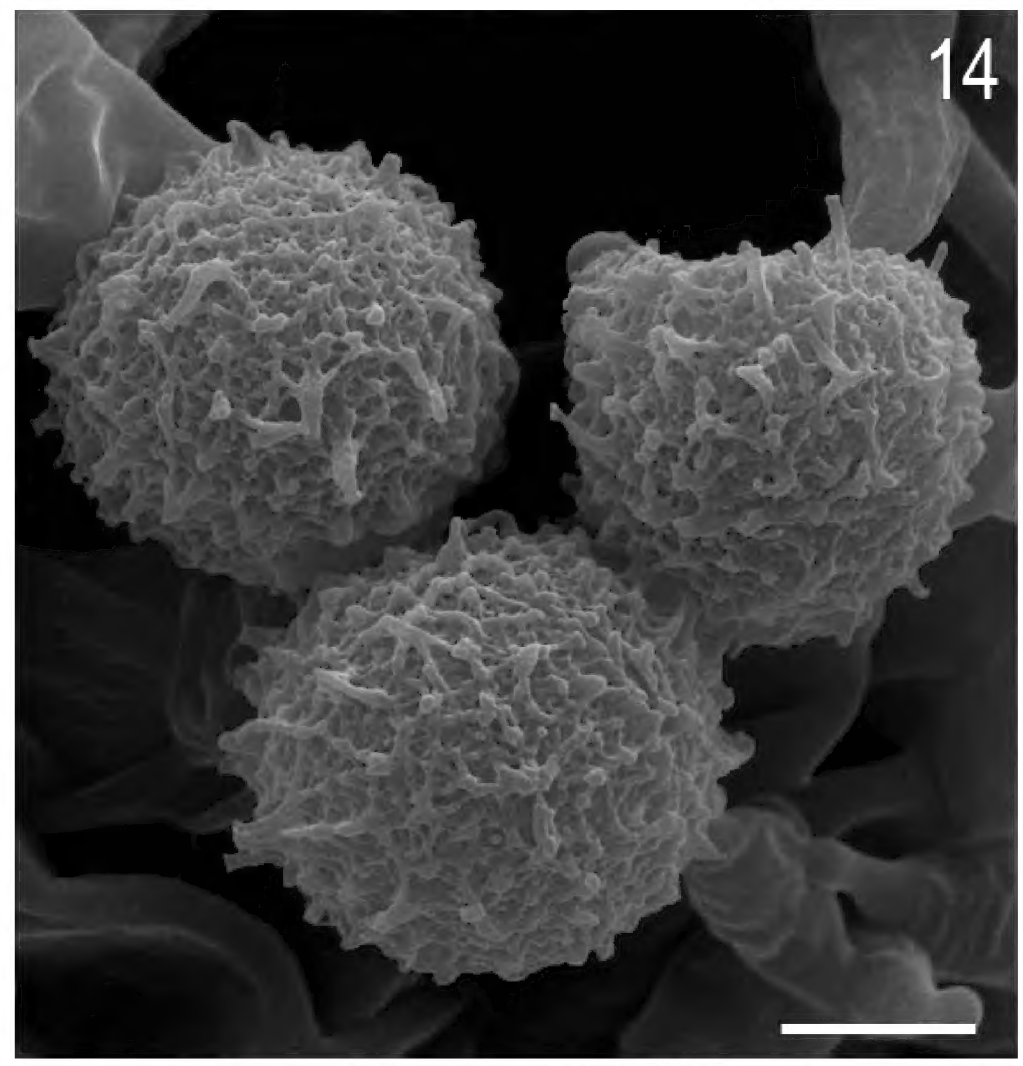


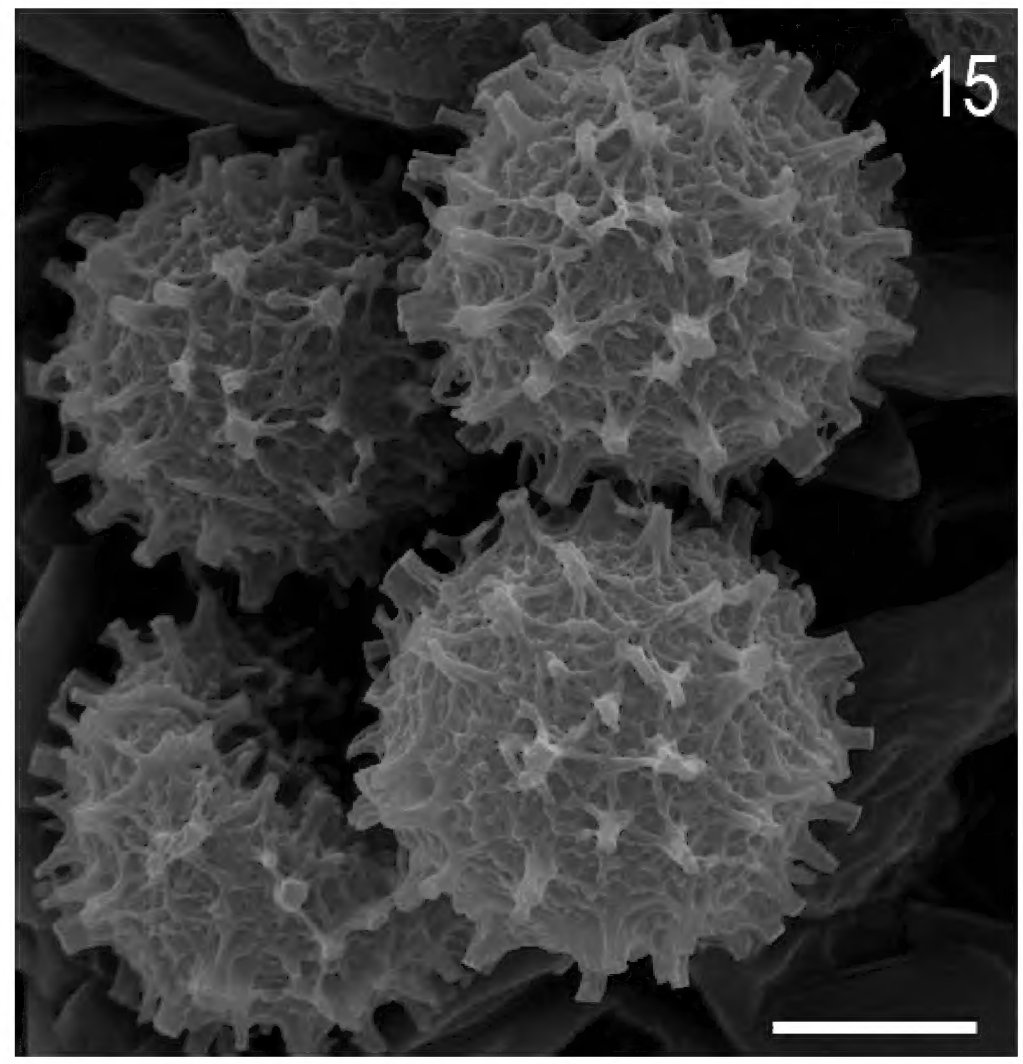
Figures 5–13. Microscopic features of *Neopaxillus echinospermus*. **5:** Basidiospores (under KOH). **6:** Section of lamella exhibiting crowded cheilocystidia (KOH). **7:** Catenulate cheilocystidia (Congo red). **8:** Cheilocystidia (Congo red). **9:** Basidium (Congo red). **10:** Pileipellis hyphae (KOH). **11**: Caulocystidia (Congo red). **12:** Clamp connection (Congo red). **13**: Oleiferous hyphae from stipe trama (Congo red). All photos by A. Silva-Filho (5,6,8,10 from HCP 705; 7,9,11,12,13 from HCP 704). Scale bar =20 µm.

becoming yellowish brown (5D5–5E8) when dry, margin incurved to plane, even, slightly undulate or lobate, non-striate. Lamellae decurrent to arcuate, subdistant with lamellulae of three lengths (Figure 2), margin straight, smooth to slightly crenulate, concolorous with sides, white (1A1), yellowish white (1A2) when young, becoming light brown (5D6) at maturity, membranous slightly fleshy. Stipe 20–39 × 2–3.5 mm (base), 2.5–7 mm (apex), central, cylindrical to slightly tapered, with base clavate to sub-bulbous, smooth to slightly striate, yellowish white (1A2) with pale yellow (1A3) to brownish orange (5C3) spots, context solid, becoming hollow at maturity. Rhizomorphs absent, but in young specimens a scanty basal mycelium can be observed. Context confluent, white (1A1) when fresh and young to yellowish white (1A2) in age or when sectioned, fleshy and soft, up to 5 mm thick. Spore print brown (6E8).

Basidiospores 6.5–9 × 6.5–8.5 µm (excluding ornamentation), Q= 1.05–1.38, Q_m= 1.13, n= 70/4/4, subglobose to globose, echinulate to warty, thick-walled, pale brown, inamyloid, commonly containing a large oil drop (Figure 5); the ornamentation is composed of short warts when younger, then distinctly spiny when fully mature, measuring 0.5–0.9 µm; under SEM, the ornamentation is composed of a densely reticulate background, from which arise columnar spines with flattened tips (Figure 15). Basidia 28.5–52 × 7.5–11 µm, narrow clavate to clavate, with four (Figure 9), rarely two sterigmata, hyaline to pale yellowish. Pleurocystidia absent. Cheilocystidia 18.5–32 (–44.5) × 6.5–16 µm, numerous and crowded, becoming the gill edge sterile (Figure 6), clavate, pyriform or sphaeropedunculate, a few lageniform, cylindrical to catenulate, sometimes with a short pedicel, hyaline to pale yellowish (Figures 7 and 8). Pileipellis composed of two layers: hymeniform suprapellis, composed of cylindrical, clavate with rounded apex, sometimes subcapitate elements, 33.5–49 × 6.7–10 µm, slightly incrusted (Figure 10), pale brown and dextrinoid; subpellis composed of radially arranged filamentous hyphae, walls thin, slightly incrusted or smooth, pale brown, 3–5.7 µm wide. Pileus trama of radially arranged hyphae, 3.4–8 µm diam., hyaline. Lamella trama subregular, formed of filamentous and narrow to slightly inflated hyphae, hyaline, 3.5–7 µm wide. Subhymenium composed of hyaline thin hyphae, 2–2.5 µm diam. Stipitipellis composed of smooth, hyaline and slightly incrusted hyphae, 2.8–7.3 µm wide, bearing bunches of caulocystidia. Caulocystidia 27–56 × 5–14 µm, in fascicles on stipe surface, cylindrical, clavate (Figure 11) and sphaeropendiculate, thin-walled and hyaline. Oleiferous (thrombopleurous) hyphae (Figure 13) observed in the pileus, lamella trama, and stipe tissue. Clamp connections present in all septa (Figure 12).

Habitat: In the forest, terricolous, among litterfall, solitary to gregarious or subcaespitose, in small clusters, on bare soil.





Figures 14 and 15. SEM micrographs of the basidiospores of *Neopaxillus echinospermus* (HCP 704). **14:** Basidiospores with partially developed ornamentation. **15:** Mature basidiospores with a fully developed ornamentation. Scale bar =2 µm.

Known distribution: In tropical (Amazon Rain Forest and Yungas, Bolivia) and subtropical (Bosque Chaqueño and Yungas in Argentina, Bosque Chaqueño in Paraguay, Atlantic Forest in Brazil) regions of South America (Singer 1964). In Brazil, it has been reported only for Dense Ombrophilous Forests and Mixed Ombrophilous Forest in São Paulo and Paraná states (de Meijer 2008; Watling and de Meijer 1997). The probable type locality of *N. echinosporus*, as discussed later, is within the Pampa

biome of Brazil. It is the first record of the species from the Seasonal Deciduous and Semideciduous Forests. Figure 1 presents the known distribution of the species.

Examined specimens: BRAZIL. PARANÁ: Palotina: Parque Estadual São Camilo, 19/V/2010, Ferreira A. J. & Souza D. (HCP 339); *ibid*, 02/III/2015, Silva-Filho AGS-189 (HCP 707); *ibid*, 27/IV2015, Silva-Filho AGS-337 (HCP 708); *ibid*, 15/VI/2015, Silva-Filho AGS-482 (HCP 709); Terra Roxa: RPPN: Fazenda Açú, 24/II/2015, Silva-Filho AGS-141 (HCP 704); *ibid*, Silva-Filho AGS-144 (HCP 705); *ibid*, Silva-Filho AGS-150 (HCP 706). RIO GRANDE DO SUL: Itaara: Parque Pinhal, 04/V/2007, V.G. Cortez 082/07 (HCP 710); Santa Cruz do Sul: UNISC, 13/III/1996, M.A. Sulzbacher 167 (HCB 16676); Santa Maria: Morro Mariano da Rocha, 23/III/2007, V.G. Cortez 028/07 (HCP 711); Três Barras, 14/03/2008, V.G. Cortez 052/08(HCP 712); and 063/08 (HCP 713).

The literature presents some controversial morphological data on *Neopaxillus echinospermus*. In the original description, Singer (1948) described the species as without cystidia, but with cystidioles on the gill margin, clamp connections in all septa, and pileipellis composed of a trichoderm-palisade. On the other hand, Watling and de Meijer (1997) and Watling and Aime (2013) reported specimens from Paraná state without any type of cystidia, absence of clamp connections, and pileipellis formed of filamentous, somewhat incrusted, and irregularly arranged hyphae. De Meijer (2008) presented a detailed description and illustrations of *N. echinospermus* and, in agreement to our examined material, he described the presence of both cheilocystidia and clamp connections. Vizzini et al. (2012), on discussing *N. dominicanus* Angelini & Vizzini, also examined specimens of *N. echinospermus* from Paraná (collected by A.A.R de Meijer) and not only observed cheilocystidia but also inconspicuous pleurocystidia, which we have not found in our materials.

All examined samples from Paraná and Rio Grande do Sul exhibited conspicuous clamp connections (Figure 13) and cheilocystidia (Figures 6–8), with variable morphology, including presence of catenulate cheilocystidia (Figure 7); the pileipellis of these mushrooms also presented the same trichoderm-palisadoderm pattern described by Singer (1948), which is mainly composed of clavate or (sub-) cylindrical elements. It is probable that older specimens can be more difficult to note the presence and position of such structures, but when fresh collections are examined they are conspicuous. In addition, we also noted the presence of oleiferous (thrombopleurous) hyphae in all parts of the basidiomata (Figure 13), a feature only mentioned in the recently described *N. dominicanus* (Vizzini et al. 2012). The great variation on the color of pileus is also illustrated here (Figures 3 and 4), however this feature alone is of poor taxonomic value, since the basidiomata persist for several days and are subject to numerous environmental factors that change the basidiomata colors.

The probable mycorrhizal status of this mushroom is to be determined, since the genus was considered in the past close to Paxillaceae and Cortinariaceae, two families with numerous ectomycorrhizal genera. Our field observations suggest that this species would not be a mycorrhizal fungus, because when the basidiomata are carefully removed from the substrate, we have not noticed the presence of rhizomorphs or other morphological evidences of this partnership. No molecular or culture studies were performed to determine (or not) the mycorrhizal status in *Neopaxillus* members, but as previously pointed, the absence of typical plant partners in this part of Atlantic Forests, as well the current placement of the species in the non-mycorrhizal family Crepidotaceae are strong evidences suggesting saprotrophic condition (Aime et al. 2005; Vizzini et al. 2012; Watling and Aime 2013).

Neopaxillus echinospermus is known from South American tropics and subtropical regions (Argentina, Paraguay, Bolivia, and Southern and Southeastern Brazil); reports from Central America and México were considered by Vizzini et al. (2012) as misidentifications of *N. dominicanus*. In Brazil, the species has been previously reported from Ombrophilous Forests, especially the Mixed Ombrophilous Forest from Southern (de Meijer 2008, from Paraná) and Dense Ombrophilous Forest in Southeastern (Spegazzini 1889, from São Paulo). The type locality of *N. echinosporus* (Singer 1948) is not precise; the material was collected by J. Rick in a place called Couto which, according to Watling and de Meijer (1997), may to represent a locality near the municipality of Rio Pardo in the central region Rio Grande do Sul state. If correct, this area belongs to the Pampa biome of southern Brazil, and comprises swampy and grassland vegetation, composed mainly of native grasses, with tree vegetation found only of riparian forest (Silva et al. 2011). With the new findings, the distribution of this species is now expanded to the Seasonal Semideciduous Forests from the state of Paraná and Seasonal Deciduous Forests of Rio Grande do Sul state, both in Southern Brazil. New reports of *N. echinospermus* are expected from other areas of Central Brazil and South America, requiring future mycological fieldwork on these areas.

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Author contributions: AGSS-F collected the samples, described the morphological characteristics of the specimens, took the photos, and produced the figures and map. GC and VGC, collected and identified samples, contributed to the introduction, discussion, literature and article review.

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